Appendix A

Using Hyperaccumulator Plants to Phytoextract Soil Ni and Cd

Rufus L. Chaney[a,*], J. Scott Angle[b,h], Marla S. McIntosh[b,h], Roger D. Reeves[c,h], Yin-Ming Li[d], Eric P. Brewer[d], Kuang-Yu Chen[b], Richard J. Roseberg[e], Henrike Perner[f], Eva Claire Synkowski[b], C. Leigh Broadhurst[b], S. Wang[b], and Alan J. M. Baker[g,h]

[a] USDA-Agricultural Research Service, Animal Manure and By-Products Lab, Beltsville, Maryland, USA, 20705. E-mail: chaneyr@ba.ars.usda.gov
[b] University of Maryland, College Park, MD, USA
[c] Institute of Fundamental Science-Chemistry, Massey University, Palmerston North, NZ, USA
[d] Viridian LLC, Houston, TX, USA
[e] Oregon State University, Central Point, OR, USA
[f] Institute for Plant Nutrition, Hohenheim University, Germany
[g] University of Melbourne, Melbourne, Australia
[h] Phytoextraction Associates LLC, Baltimore, MD, USA

* Author for correspondence and reprint requests

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Two strategies of phytoextraction have been shown to have promise for practical soil remediation: domestication of natural hyperaccumulators and bioengineering plants with the genes that allow natural hyperaccumulators to achieve useful phytoextraction. Because different elements have different value, some can be phytomined for profit and others can be phytoremediated at lower cost than soil removal and replacement. Ni phytoextraction from contaminated or mineralized soils offers economic return greater than producing most crops, especially when considering the low fertility or phytotoxicity of Ni rich soils. Only soils that require remediation based on risk assessment will comprise the market for phytoremediation.

Improved risk assessment has indicated that most Zn + Cd contaminated soils will not require Cd phytoextraction because the Zn limits practical risk from soil Cd. But rice and tobacco, and foods grown on soils with Cd contamination without corresponding 100-fold greater Zn contamination, allow Cd to readily enter food plants and diets. Clear evidence of human renal tubular dysfunction from soil Cd has only been obtained for subsistence rice farm families in Asia. Because of historic metal mining and smelting, Zn + Cd contaminated rice soils have been found in Japan, China, Korea, Vietnam and Thailand. Phytoextraction using southern France populations of *Thlaspi caerulescens* appears to be the only practical method to alleviate Cd risk without soil removal and replacement. The southern France plants accumulate 10–20-fold higher Cd in shoots than most *T. caerulescens* populations such as those from Belgium and the UK. Addition of fertilizers to maximize yield does not reduce Cd concentration in shoots; and soil management promotes annual Cd removal. The value of Cd in the plants is low, so the remediation service must pay the costs of Cd phytoextraction plus profits to the parties who conduct phytoextraction. Some other plants have been studied for Cd phytoextraction, but annual removals are much lower than the best *T. caerulescens*. Improved cultivars with higher yields and retaining this remarkable Cd phytoextraction potential are being bred using normal plant breeding techniques.

Key words: Zinc, *Thlaspi caerulescens*, *Alyssum murale*

Introduction

Phytoextraction of soil metals has constraints that need to be considered by anyone who plans research or commercial application. Even with high biomass yields, crop plants cannot remove enough metals per year to achieve useful phytoextraction (Table I). Rather, because they tolerate about 100-fold higher metals in leaves than do crop plants, hyperaccumulators are necessary for successful phytoextraction. And although many hyperaccumulators are small and grow slowly, some have high yield potential, are perennial, and are tall enough for mechanical harvest. Our success in developing a commercial Ni phytomining technology with *Alyssum murale* shows what is possible (Li *et al.*, 2003). Further, addition of chelators such as EDTA to conduct "induced phy-

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toextraction" with *Brassica juncea* or other species is unacceptable for *in situ* phytoextraction. Perhaps it could be acceptable *ex situ*, but if one pays to remove soil from the field to conduct *ex situ* phytoextraction, one would take it directly to the landfill rather than conduct an expensive EDTA-induced phytoextraction treatment. Several field tests found EDTA addition to firing range soils rich in Pb quickly caused Pb leaching to ground water and projects were cancelled. Further, adding 10 mmol EDTA kg^{-1} soil costs about \$ 30,000 ha^{-1}, a large annual cost.

Another consideration is whether the soil contamination comprises environmental risk. By careful study of the agronomy and human nutrition aspects of Cd risks to humans, R. L. Chaney, P. G. Reeves and collaborators have shown that only subsistence rice farmers who eat locally produced polished rice grain for their lifetime are at risk from soil Cd for nearly every Cd contaminated site (Chaney *et al.*, 2004). Nearly every Cd contaminated site had a geogenic Zn mine or smelter source with about 100 g Zn per 1 g Cd. Only rice allows enough uptake and transfer of Cd to grain without Zn that the Cd can be a risk to humans (Simmons *et al.*, 2003; Chaney *et al.*, 2004). Deficiency of Fe and Zn in rice subsistence diets causes absorption of Cd to increase 10–20-fold normal (Reeves and Chaney, 2002). Foods other than rice (shellfish, sunflower kernels, durum wheat) have not caused unusual Cd absorption when tested in humans apparently because they supply Fe, Zn, and phytate, all of which strongly inhibit Cd uptake (Reeves and Chaney, 2002; Vahter *et al.*, 1996).

Unfortunately, Cd contamination of rice soils from suspended solids in irrigation water has occurred in many locations in Asia, and the soils have sufficient soil Cd to cause human Cd disease (*e.g.* Kobayashi, 1978; Nogawa, 1981; Cai *et al.*, 1990). Additional contaminated sites are known in rice production areas of Korea, Thailand and Vietnam. Zn–Pb mining or smelting, which could contaminate rice or tobacco production soils, will require extraordinary management to prevent crop contamination. These two crops allow transfer of soil Cd to humans in highly bioavailable forms. High exposures can cause proximal tubular disease of the kidney by age 50. Because tobacco accumulates Cd in leaves, and tolerates Zn somewhat, one can grow apparently normal tobacco with 25–50 mg Cd kg^{-1} dry weight. Smoking the equivalent of one pack of cigarettes made from such tobacco per day can contribute to or cause Cd renal tubular disease (Cai *et al.*, 1990).

All other Cd contamination as part of geogenic Zn contamination has not been shown to comprise risk to highly exposed humans. Garden foods grown in soils with 100 mg Cd and 10,000 mg Zn kg^{-1} have not caused unusual Cd absorption in humans (see summary in Chaney *et al.*, 2004). Thus, nearly all the Zn + Cd contaminated soils in Europe and the USA do not require Cd phytoextraction. Further, *in situ* remediation and ecosystem restoration of Zn, Cu, Ni and Pb contaminated soils using limestone equivalent plus Fe and P rich organic amendments (biosolids, composts) is very cost-effective and persistent (*e.g.* Ryan *et al.*, 2004).

Thus phytoextraction needs to be applied to all the geogenic Zn + Cd contaminated rice and tobacco land, and to cropland which is contaminated by sources with high Cd:Zn ratio. High Cd:Zn ratio allows plants to contain much higher Cd levels before Zn phytotoxicity reduces yields. Other Cd contaminated soils (*i.e.*, some soil amended with high Cd biosolids before modern regulations, high Cd fertilizers, Cd-rich marine shale derived soil, and sites contaminated by certain Cd pigment or PVC stabilizer chemical factories or Ni-Cd battery factories) may contain Cd with high Cd:Zn ratio, which may also require remediation to protect humans.

New understanding of Cd risk to humans

Based on new data from meta-analysis of Japanese epidemiological experiments, no one in Sweden or Belgium has been harmed by soil Cd transfer to food crops. After considering different points of view regarding the concentration of Cd in urine which may serve as a diagnostic level to indicate human renal tubular dysfunction, Ikeda *et al.* (2003) conducted a meta-analysis of the epidemiological studies in Japan where high prevalence of renal tubular disease occurred in subsistence rice farm families. They reported a urine Cd threshold at least as high as 10 μg Cd g^{-1} creatinine before clinically significant tubular dysfunction began to occur. This contrasts sharply with suggestions from Swedish and Belgian researchers that levels of urinary Cd as low as 2.5 μg Cd g^{-1} creatinine may possibly indicate early stages of renal tubular dysfunction. Research has shown that

urinary levels of low molecular weight proteins (diagnostic for tubular dysfunction) increases with age, is influenced by exercise and other factors. For example, persons over 60 years of age with Cd-induced renal tubular disease have urinary levels of β_2-microglobulin of 100,000–200,000 μg l^{-1}. For a young healthy population, mean secretion is about 100 μg l^{-1}. Use of the 95% upper confidence level for the mean of young populations has no relationship with diagnostic levels for Cd-induced renal tubular dysfunction. Diagnostic thresholds of 1000 μg l^{-1} or higher are commonly used (*e.g.*, Ikeda *et al.*, 2003)

With this reanalysis of diagnostic levels of urinary Cd, Ezaki *et al.* (2003) measured Cd in diet, blood and urine and low molecular weight proteins in urine of over 10,000 Japanese urban middle-aged non-smoking women who were examined in recent years to evaluate risk to the general population from Cd in rice in Japan. Rice provided the majority of dietary Cd to these women and their Cd intakes were about half of the WHO recommended PTWI limit for dietary Cd and about double the diet Cd in Europe or North America; but no adverse Cd effects were observed.

Rice is a special case for soil Cd risk to humans

Contaminated rice fields have caused human Cd disease. Several properties of paddy rice production and characteristics of the rice plant allow soil Cd to reach rice grain in a bioavailable form. Other crops grown in aerobic soils do not allow the transfer of soil Cd to edible plant tissues in such bioavailable form. The key features which affect Cd in rice include: 1) production in flooded soils (paddy); 2) formation of sulfides and rise in soil pH to 7 or above during flooded management (prevents Zn phytotoxicity); 3) a rapid drop in soil pH when farmers ordinarily drain the fields (at flowering to maximize yields and prepare for harvest); oxidation of the reduced soil Fe, Mn, sulfide, etc., causes the rapid soil acidification and releases Cd in phytoavailable forms; 4) because rice grown in flooded soils is not harmed by normal soil acidity, farmers often fail to apply limestone to maintain soil pH near the 6.5 recommended for vegetable crops; low soil pH of drained/oxidized paddy soils promotes Cd uptake to rice grain; 5) Cd is rapidly moved to grain during grain filling without Zn or Fe; and 6) polishing removes much of the Zn and Fe in brown rice (Chaney *et al.*, 2004; Simmons *et al.*, 2003).

Rice grain has low Zn level even on highly Cd + Zn contaminated soils. This was evident in the many field studies in Japan (Asami, 1984). The study by Simmons *et al.* (2003) illustrates these principles by comparing Cd and Zn accumulation in rice and soybean grain from adjacent fields which are cropped with both species in rotation. In aerobic soils, high Zn levels can inhibit Cd uptake by roots, and inhibit transport to shoots and storage tissues (grain, fruits, tubers). Further, Zn serves as a maximum limit on Cd accumulation in plant leaves; when Zn causes phytotoxicity, Cd is limited to the ratio of Cd:Zn in the soil (Chaney, 1993). Extensive area of rice paddy soils have become Cd contaminated; therefore inexpensive remediation methods are needed to remove the soil Cd and protect the health of the subsistence farm families who consume foods grown on the contaminated soils.

Phytoextraction concept

Soil remediation by phytoextraction of metals from contaminated soils using metal hyperaccumulator plants was first suggested by Chaney (1983). The default remediation method of soil removal/replacement is very expensive. Hyperaccumulator plant species exist in nature, which can accumulate over 1% of Zn, Ni, Co, Cu, Mn, Se, As, and Cd without causing yield reduction of the plants. The biomass with accumulated metals can be disposed in a landfill, or burned to generate biomass energy and the ash disposed or recycled by metal smelters as a new kind of metal ore.

We must domesticate these natural hyperaccumulators into "new crops" or bioengineer the hyperaccumulator properties into higher yielding plants in order to have practical phytoextraction of Cd from contaminated soils. The model would be to farm the phytoextraction crop to remove metals from soil, and make "hay" using traditional farm practices. One needs to develop all soil and plant management practices needed to produce high yields of the phytoextraction crop and maximize annual accumulation of metals in the plant shoots. One could incinerate or pyrolyze the biomass to recover energy and ash; energy could pay part of the soil remediation costs. The metals could be recovered from the biomass ash (as in phytomining of soil Ni), and marketed or disposed

safely. There is little market demand for Cd today because the use of Cd in commercial products has been greatly reduced due to the potential health risks from Cd. Cd is recovered during smelting of Zn and disposed safely. The value of Zn in the ash of a Cd phytoextraction crop may also reduce the costs of soil remediation.

Why does it take hyperaccumulator species to make phytoextraction practical?

All plants absorb metals from soils. Crop plants have "normal" metal tolerance and variable metal uptake, which means that at some metal concentration in shoots, yield will be reduced by metal phytotoxicity. Most plants suffer toxicity when leaves contain about 400–500 mg Zn kg^{-1} or Ni exceeds 50–100 mg kg^{-1} dry matter. No matter what their yield potential, crop plants cannot accumulate much Zn or Ni (Table I). Plants vary more widely in tolerance of absorbed Cd; some species have yield reduction and visible symptoms of toxicity when they contain as low as 5 or as high as 100 mg kg^{-1} dry weight. Table I shows a comparison of plant accumulation of Ni; the high yield maize suffers strong phytotoxicity by 100 mg Ni kg^{-1} when soil phytoavailable Ni is increased. However, the hyperaccumulator species *Alyssum murale* can easily accumulate 20,000 mg Ni kg^{-1} (2%) dry weight with no yield reduction; we have grown healthy *A. murale* with 2.7% Ni in shoots on Oregon, USA, serpentine soils (Chaney *et al.*, 2000; Li *et al.*, 2003). Further, the mean cost of Ni at the London Metals Exchange for the last 20 years has been about \$ 9 kg^{-1}; the current price is nearly \$ 15 kg^{-1}.

A team of scientists from USDA-ARS, University of Maryland, Sheffield University, UK, Oregon State University and Viridian LLC developed a commercial Ni phytoextraction/phytomining technology by domesticating tall *Alyssum* species with highly effective natural Ni hyperaccumulation (*e.g.*, *A. murale*). Seeds were collected from diverse sites across southern Europe and evaluated under uniform conditions in the field. Laboratory and field experiments were conducted to identify agronomic management practices needed to produce high yields in severely infertile serpentine soils in Oregon, USA, or in smelter contaminated soils at Port Colborne, Ontario, Canada. Low fertilizer applications (*Alyssum* is adapted to serpentine soils which are very low in phytoavailable P and Ca) greatly increased annual yield without decreasing shoot Ni concentration. Weed control chemicals for *Brassica* species were very effective in limiting plant competition. Remarkably, plant accumulation of Ni was improved at higher pH values, which caused lower soluble Ni levels in the soil; but plant accumulation of Zn, Mn and Co followed the usual pattern of reduced shoot levels at higher soil pH (Kukier *et al.*, 2004). High levels of Fe oxides in serpentine soils limited this effect to about pH 6.5, while smelter contaminated farm soils had maximum annual Ni uptake at pH 7.5 (Kukier *et al.*, 2004). Biomass was cut at early flowering to prevent loss of leaf biomass (richer in Ni than stems) and was baled mechanically. Subsequently *Alyssum* biomass ash was smelted in a standard industrial facility at Sudbury, Ontario, and Ni metal was recovered very effectively. *Alyssum* biomass appears to be the best ore for Ni ever produced; elements in plant biomass do not interfere with recovery of Ni by smelter operations. Improved cultivars were bred by traditional plant breeding methods considering the self-incompatible nature of *A. murale*. Patents on this technology (*e.g.*, Chaney *et al.*, 1998) are licensed to Viridian LLC which is marketing Ni phytoextraction at several locations.

Table I. Crop and hyperaccumulator plant models for Ni phytomining. The second *Alyssum murale* listing presumes that plant breeding has been used to develop a commercial cultivar for phytomining Ni (Li *et al.*, 2003). Maize is modeled as a forage crop; ash weight is about 5–10% of dry weight.

Assume soil contains 2500 mg Ni kg^{-1} = 10,000 kg Ni (ha-30 cm)$^{-1}$					
Species	Yield [t ha^{-1}]	Ni in the crop [mg kg^{-1}]	[kg ha^{-1}]	(% of soil Ni)	Ash–Ni (%)
Maize (100% normal)	20	2	0.04	0.0004	0.008
Maize (50% normal yield)	10	100	1	0.01	0.20
Wild *Alyssum*	10	20,000	200	2.0	20–40
Alyssum cultivar	20	30,000	600	6.0	25–50

One topic often raised about phytoextraction is whether wildlife will be harmed by the metals in hyperaccumulator biomass. In the case of the Ni phytomining technology, observations of livestock (sheep, cows, goats) and wildlife (deer, rabbits) grazing in fields with dense *Alyssum* species showed that these animals do not graze the *Alyssum* biomass. Examination of *Alyssum* biomass indicates that the dense trichomes make the plants unpalatable. Further, the seeds of *Alyssum* are very small and are not a useful food for wildlife.

Development of Cd phytoextraction technology

The approach our team used to develop Cd phytoextraction technology is based on our understanding of the science and the potential market for Cd and Zn phytoextraction. Because the Zn and Cd accumulated in biomass have much lower value than Ni, the value of metal in the biomass will not drive development and use of this technology. Biomass energy can reduce the cost of soil Cd cleanup, but is unlikely to make Zn + Cd phytoextraction profitable by phytomining. Rather, the value of the cleanup will drive the market. Risk from Zn can be controlled by soil pH management, but for Cd, risk from rice soils, tobacco soils, and soils with high Cd:Zn ratio must be remediated to be able to produce crops safe for consumption. Most of the Cd phytoextraction market will be in paddy rice land contaminated by emissions of Zn–Pb–Cu–Ag mining and smelting. It is now clear that paddy rice land in Japan, China, Korea, Thailand and Vietnam have been contaminated enough to require remediation or change in land use.

We adopted the "agricultural paradigm" to develop a Cd phytoextraction technology. Table II contains models for Zn and Cd phytoextraction by crop and hyperaccumulator species. The value of southern France genotypes of *Thlaspi* is evident. We selected *Thlaspi caerulescens* for development because it was the largest Zn hyperaccumulator which also accumulated Cd. The 'Prayon' population was initially studied by researchers because Alan Baker shared collected seed. Some other species which can accumulate Cd exist (Table III), but were even smaller than *Thlaspi* (*e.g.*, *Arabidopsis halleri*, *Arabis gemmifera*), or did not accumulate favorable levels of Cd (*Dichapetalum gelonioides*).

After our initial studies to understand the potential of *Thlaspi* to accumulate soil and solution Cd and Zn (Brown *et al.*, 1994, 1995), we evaluated 20 different *Thlaspi* genotypes in nutrient solutions and a smaller subset in contaminated soils. The genetic screening in nutrient solutions demonstrated high accumulation of Cd by southern France populations compared to other popula-

Table II. Estimated removal of Zn and Cd in biomass of corn (*Zea mays* L.) at full yield or with 50% yield reduction due to Zn phytotoxicity on contaminated soil, or *Thlaspi caerulescens*, either the 'Prayon' ecotype, or an improved cultivar with higher yield and 10-times higher Cd accumulation. Annual Cd phytoextraction would be expected to decline as soil Cd was depleted.

Zn: Presume soil has 2000 ppm Zn = 4000 kg Zn (ha-15 cm)$^{-1}$					
Crop	Yield [t ha^{-1}]	Zn in crop [mg kg^{-1}]	[kg ha^{-1}]	(% of soil Ni)	Zn in ash (%)
Corn (normal soil)	20	50	1.0	0.0025	0.10
Corn-Zn phytotoxicity	10	500	5.0	0.0125	0.50
Thlaspi	5	25000	125.	3.12	40
Improved *Thlaspi*	10	25000	250.	6.25	40

Cd: Presume soil has 20 ppm Cd = 40 kg Cd (ha-15 cm)$^{-1}$					
Crop	Yield [t ha^{-1}]	Cd in crop [mg kg^{-1}]	[kg ha^{-1}]	(% of soil Cd)	Cd in ash (%)
Corn	20	0.5	0.01	0.025	0.001
Corn	10	5	0.05	0.125	0.005
Thlaspi 'Prayon'	5	200	1.0	2.5	0.40
Thlaspi S. France	5	2000	10.0	25.	4.00
Thlaspi Improved	10	2000	20.0	50.	4.00

Table III. Zn and Cd in shoots of plant species evaluated for Cd phytoextraction when grown on soils with geogenic Zn + Cd contamination (Chaney *et al.*, 2002).

Species	Max. Zn (% DW)	Max. Cd [mg kg^{-1} DW]
Arabidopsis halleri	1.4	100.
Thlaspi caerulescens (Prayon)	2.7	250.
Thlaspi caerulescens (S. France)	2.5	2500.
Dichapetalum gelonioides	3.0	2.1
Athyrium yokoscense	0.64	165.
Arenaria patula	1.31	238.
Sedum alfredii Hance	1.80	180
Willow/poplar leaves		
Upland rice cultivars	0.04	40.

tions (Fig. 1) (Li *et al.*, 1996). A similar outcome was observed when we grew *Thlaspi* genotypes in smelter contaminated field plots at Palmerton, PA (Fig. 2) (Li *et al.*, 1997). We concluded that natural variation in Cd accumulation by *Thlaspi* populations would support breeding of improved cultivars useful for practical Cd phytoextraction. Strong promise was also reported by Keller and Hammer (2004) who grew *T. caerulescens* in several contaminated fields.

Phytoextraction Associates LLC is working with USDA-ARS to develop and commercialize improved *T. caerulescens* cultivars with higher yields and the high Cd:Zn accumulation of southern France genotypes. Based on the findings of Reeves *et al.* (2001) who analyzed *T. caerulescens* collected across Europe, the southern France genotypes are of special importance. Fig. 3 shows the Cd and Zn levels in 'Prayon', Viviez and St. Félix-de-Pallières genotypes. In the study of Perner *et al.* (2005), eight plants were grown from seed of each of 25 mother plants collected at these southern France Zn–Cd-contaminated sites. The wide variation among siblings [due to mixed inbreeding and out-

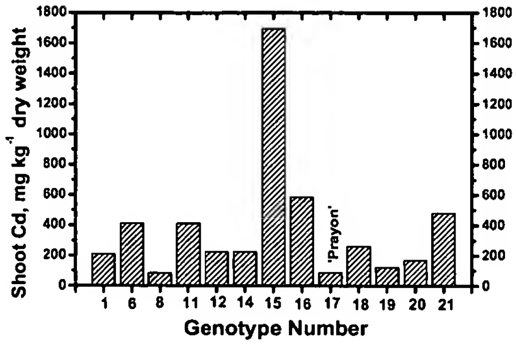

Fig. 2. Cd accumulation into shoots of *Thlaspi caerulescens* ecotypes grown in the field at Palmerton, PA, where soil contains 15,000 mg Zn and 150 mg Cd kg^{-1} dry weight (Li *et al.*, 1997). The 'Prayon' ecotype is marked. All of the much higher Cd ecotypes came from the southern French populations (see Reeves *et al.*, 2001).

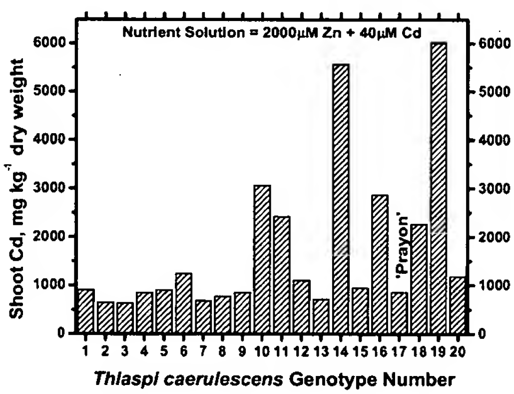

Fig. 1. Cd accumulation of 20 accessions of *Thlaspi caerulescens* when grown in nutrient solutions containing 2000 μM Zn plus 40 μM Cd (Li *et al.*, 1996; Chaney *et al.*, 2000).

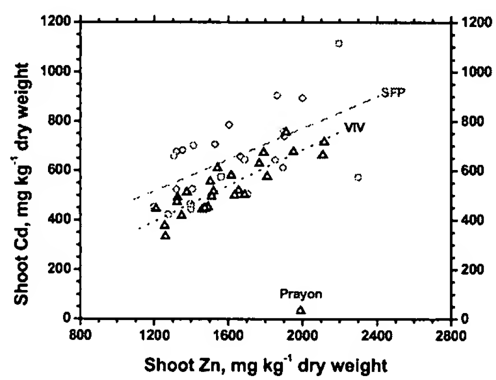

Fig. 3. Relationship of shoot Cd and Zn among *Thlaspi caerulescens* populations collected at three locations: Prayon, Belgium; St. Félix-de-Pallières (SFP), France; and Viviez (VIV), France (each point is the mean for 7–8 replicate sibling plants). Plants were grown for 62 d in pots of soil with 47.6 mg Cd kg^{-1} and 821 mg Zn kg^{-1} due to application of high Cd:Zn biosolids for many years (Perner *et al.*, 2005).

crossing (Synkowski *et al.*, 2005)] suggests that Cd hyperaccumulation is a quantitative trait, while the southern France genotypes appear to have a single gene which causes 10-fold higher Cd accumulation than 'Prayon' genotypes. Although more research is being conducted to understand these genetic differences, it is clear that normal plant breeding can be used to develop cultivars with higher yield and high annual Cd removal needed to achieve remediation of soils which require Cd remediation to protect human health.

Where are there possible commercial applications for Cd phytoextraction?

We noted above that paddy rice land in Japan, China, Korea, Vietnam and Thailand has been shown to be contaminated by geogenic Zn + Cd sources. Recent evaluation of the contamination of rice fields in Japan suggests that 500,000 ha would need Cd remediation if the Cd limit for marketed rice were set at 0.2 mg kg^{-1}, with about 100,000 ha needing remediation if the rice Cd limit is 0.4 mg kg^{-1}. The improved understanding of Cd effects on kidney function reported by Ikeda *et al.* (2003) and new population studies reported by Horiguchi *et al.* (2004) indicate that with present nutritional status of Japanese farm families, setting the rice limit at 0.4 mg Cd kg^{-1} would provide the needed protection for the most sensitive and exposed individuals.

One can estimate the cost of soil Cd phytoextraction needed for rice soils which produce brown rice with Cd levels greater than 0.4 mg kg^{-1} in Japan by multiplying the area times the cost per ha [100,000 ha · \$ 2,500,000 ha^{-1}] = \$ 250 billion if the traditional soil removal and replacement approach were used as shown in the remediation of about 646 ha in Japan in the 1980s (Iwamoto, 1999). The extreme cost of remediation of this area by soil removal and replacement has delayed government decisions on soil Cd remediation. Fortunately, phytoextraction of this Cd would cost < 1% of soil removal. Rice farmers could be paid to produce high Cd *T. caerulescens* biomass during a short clean up period, and be paid based on the amount of Cd in delivered biomass (mass times concentration) to encourage best phytoextraction practice for production of the phytoextraction crop.

Localization of hyperaccumulated metals in leaves

Several approaches have shown that Zn and Cd are accumulated in vacuoles of epidermal cells of *Thlaspi caerulescens* (Küpper *et al.*, 1999; Frey *et al.*, 2000). Some species appear to have highly selective storage of hyperaccumulated metals in specific cells, while others have more general accumulation in most leaf cells, or even have some metals in the cell wall free space. Besides the practical development of phytoextraction technology discussed above, our team has reported research on a role of soil microbes in metal hyperaccumulation, and on localization and chemical forms of metals in shoots of Ni hyperaccumulators (Broadhurst *et al.*, 2004). Reports on X-ray absorption spectroscopy examination of forms of Ni in soils in smelter contaminated soils, and Ni and other elements in *Alyssum* tissues have been conducted by graduate students of D. L. Sparks and will soon be reported. Together this work on localization has shown that trichome cells have exceptional accumulation of Ni and Mn in vacuoles compared to other epidermal cells, and that trichomes tissue exterior to the cuticle have high Ca but very low Ni. The unexpected high accumulation of Ni and Mn in the vacuoles of the cells from which trichomes are elaborated indicates greater complexity of cellular transport mechanisms than previously recognized. Whether different proteins or different expression of transport proteins in specific cells is the basis for this highly specific cellular accumulation remains unknown.

Future of phytoremediation

In order to achieve practical phytoextraction of soil metals, hyperaccumulator plants are necessary. Thus natural hyperaccumulators offer an important tool for inexpensive soil decontamination for those elements which plants hyperaccumulate. To date, useful natural hyperaccumulators of Ni, Co, Zn, Cd, As, and Se have been identified which yield enough annual accumulation of metals to achieve soil remediation goals. Chromate can be remediated by plant root reduction to chromic (one form of phytoremediation). Plants which phytovolatilize Hg^0 have been bioengineered, but public acceptance is weak; further development of plants which accumulate Hg in shoots may offer Hg phytoextraction acceptable to the public.

Good science is the only way to make phytoextraction into the powerful tool for soil remediation that remains promising today. Because soils often have multiple element contamination, phytoextraction cultivars must tolerate other metals. In the case of soils with geogenic Cd–Zn contamination, testing candidate plants for uptake of Cd without normal co-contaminating Zn may suggest a plant offers useful Cd removal, but without the selective 10-fold higher Cd (and Cd:Zn ratio) accumulation of the southern France *T. caerulescens* genotypes, annual Cd removal is severely limited by the Zn tolerance (*e.g.*, Indian mustard, Vetiver grass, etc.).

Only Se hyperaccumulators have the ability to selectively accumulate Se from soils, which have high levels of both sulfate and selenate. And the Se in phytoremediation biomass may be useful as a Se feed-supplement for livestock (Banuelos and Mayland, 2000). Most livestock feeds require Se supplementation because most soils have low levels of Se. But *Brassica rapa* (canola) and other species used for Se phytoremediation may be harvested as described for Ni phytomining, and sold for replacement of chemical salts of Se presently used. Because the Se is in the organic form in these plants, it provides a better source than the selenate commonly used. Further, ground Se-rich biomass would give a more even distribution of Se throughout a batch of feed than can be obtained with selenate addition.

Phytochelatins play no significant role in hyperaccumulation or even metal tolerance of natural plants (Schat *et al.*, 2002). And repeated claims that all natural metal hyperaccumulator species grow slow and have low biomass is clearly incorrect based on our development of commercial Ni phytomining technology (Li *et al.*, 2003). Natural hyperaccumulators may be difficult to domesticate for needed commercial clean up for some elements (*e.g.*, Cu, Pb), so *in situ* remediation or phytostabilization may be the method which achieves these goals. Ultimately sterile hyperaccumulator hybrids may be the most acceptable strategy for development of commercial cultivars, but this would take many years and much investment. Much challenging work and great opportunity remains to develop commercial phytoextraction services.

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